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INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

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| Applicant's or agent's file reference | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA416) | |
| International application No. PCT/EP 03/06763 | International filing date (day/month/year) 26.06.2003 | Priority date (day/month/year) 16.07.2002 |
| International Patent Classification (IPC) or both national classification and IPC C12N15/82 | | |
| Applicant UNILEVER N.V. et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I Basis of the opinion
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

| | |
|--|---|
| Date of submission of the demand 27.12.2003 | Date of completion of this report 22.10.2004 |
| Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016 | Authorized Officer Bucka, A Telephone No. +31 70 340-2279 |



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP 03/06763

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-43 as originally filed

Sequence listings part of the description, Pages

1-8 received on 24.10.2003 with letter of 24.10.2003

Claims, Numbers

1-8 filed with telefax on 05.08.2004

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.: 9-25
- the drawings, sheets:

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5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

the entire international application,

claims Nos. 1-4,6,7
because:

the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 1-4,6,7 are so unclear that no meaningful opinion could be formed (*specify*):
see separate sheet

the claims; or said claims Nos. 1-4,6,7 are so inadequately supported by the description that no meaningful opinion could be formed.

no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

the written form has not been furnished or does not comply with the Standard.

the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | |
|-------------------------------|-------------|-----|
| Novelty (N) | Yes: Claims | 5,8 |
| | No: Claims | |
| Inventive step (IS) | Yes: Claims | |
| | No: Claims | 5,8 |
| Industrial applicability (IA) | Yes: Claims | 5,8 |
| | No: Claims | |

2. Citations and explanations

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The following documents (D) are considered to be relevant to this application:

- D1: WO 01 31027 A (HARKER MARK ; HOLMBERG NIKLAS (GB); SAFFORD RICHARD (GB); UNILEVER) 3 May 2001
- D2: WO 02 42477 A (HARKER MARK ; HOLMBERG NIKLAS (GB); SAFFORD DICK (GB); UNILEVER PLC) 30 May 2002
- D3: DARNET S ET AL: 'Functional identification of sterol-4alpha-methyl oxidase cDNAs from *Arabidopsis thaliana* by complementation of a yeast erg25 mutant lacking sterol-4alpha-methyl oxidation' FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 508, no. 1, 9 November 2001, pages 39-43.
- D4: BARD M ET AL: 'Cloning and characterization of ERG25, the *Saccharomyces cerevisiae* gene encoding C-4 sterol methyl oxidase.' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 93, no. 1, 1996, pages 186-190.
- D5: PASCAL SOPHIE ET AL: 'Plant sterol biosynthesis: Identification and characterization of two distinct microsomal oxidative enzymatic systems involved in sterol C4-demethylation.' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 16, 1993, pages 11639-11654.

The amendments appear to be allowable having regard of the provisions of Article 34(2)(b) PCT.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The **claims 1 to 4, 6 and 7**, directed to methods for increasing the level of 4-desmethyl sterols in a plant, define their subject-matter only in terms of *desiderata*, in terms of results to be achieved ("increasing the enzymatic demethylation", "increasing the activity of C4SMO", "increased expression of a gene coding for C4SMO"), without giving a true technical characterization of any such method. They describe the underlying technical problem, but do neither characterize nor disclose a method for increasing the level of 4-desmethyl sterols and are therefore not allowable. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and is not supported by the description (Articles 5 and 6 PCT). Only the overexpression

of C4SMO, achieved by the transformation of tobacco with a functional, heterologous C4SMO gene, in a tobacco wild type and in a HMGR/SMT1-overexpressing background, respectively, to which claims 5 and 8 relate, is shown. No other means of "increasing the enzymatic demethylation" or increasing the "expression of a gene coding for C4SMO" (which also means the endogenous gene) are provided, which would allow the skilled person to carry out the claimed subject-matter in its broadest scope.

Therefore, since the claims are so inadequately supported by the description in the meaning of Article 6 PCT, and the application lacks sufficient disclosure in the sense of Article 5 PCT to such an extent that no meaningful opinion can be formed on the novelty, inventive step, or industrial applicability of the claimed subject-matter, the International Preliminary Examining Authority shall not go into the questions referred to in Article 33(1) PCT according to the provisions of Article 34(4)(b) PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Synopsis

The application discloses the modification of plant sterol content by the overexpression of a C4 sterol methyl oxidase. This results in a higher ratio of 4-desmethyl sterols compared to (di)methyl sterols. The enzyme was introduced in tobacco wild type plants (pages 28-34) and in a background of HMGR and SMT1 overexpressing plants (pages 35-43).

2

D2, which is considered to represent the closest prior art, describes plants overexpressing HMGR and SMT1, from which the subject-matter of claim 5 differs in that it refers to plants expressing a heterologous C4 sterol methyl oxidase. The objective technical problem to be solved by the present invention therefore is considered to be the provision of alternative means to produce plants with a higher content of 4-desmethyl sterols.

Claim 5 provides plants overexpressing a heterologous C4 sterol methyl oxidase, thereby solving the problem.

D2 is referring to the same technical problem, namely the change of the sterol composition of plants in such a way that the relative content of the "beneficial"

desmethyl sterols is increased. D2 considers the 4-desmethyl sterols as beneficial (D2, page 6), because they contribute to nutritional quality and are more desirable than for instance cholesterol. In addition, D2 is discussing a large number of enzymes, which are involved in sterol biosynthesis (D2, pages 2-4) and which can be used in the modification of sterol synthesis.

Therefore, document D2 already provides an incentive to introduce further enzymes, which are involved in the synthesis of the beneficial 4-desmethyl sterols, into plants, when attempting to modify sterol synthesis.

D3 provides the coding sequence of the *Arabidopsis* gene encoding a C4 sterol methyl oxidase, thereby providing the first of the enzymes that are responsible for the demethylation of 4,4-dimethyl sterols *in planta* and hence an obvious tool to increase the ratio of the beneficial desmethyl sterols in plants. The person skilled in the art would have used the coding region encoding said enzyme to overexpress it in transgenic plants, thereby arriving at plants with an altered content of 4-desmethyl sterols. Although D3 is not concerned with the nutritional quality of plant products, the skilled person would have recognized the suitability of the disclosed enzyme in approaches to modify sterol synthesis that are described in D2.

The assertion of the Applicant that C4SMO catalyses a rate limiting step in the production of 4-desmethyl sterols is not supported by any data. Moreover, it appears that the *absolute* level of 4-desmethyl sterols in tobacco seeds is not even altered by the expression of C4SMO (cf. description, table 7).

For the reasons outlined above, the solution proposed in **claim 5** cannot be considered as involving an inventive step (Article 33(3) PCT).

Similar objections are raised for the subject-matter of **claim 8**.

Further Comments:

- 3 The claims refer repeatedly to a comparison to the "wild type" plant. However, it is not clear, what is meant by the term "wild type", especially since some plant species (oil seeds) might have a higher sterol level than for instance tobacco (Article 6 PCT). Even within a given species, a broad variation of traits can exist.

- 4 The description shows the generation of tobacco plants transformed with a construct encoding an *Arabidopsis* C4SMO (examples 1, 2). However, it does not disclose the invention sufficiently for it to be carried out over the whole scope claimed. The application does not show how canola, rape, sunflower or soy can

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be transformed by a construct encoding C4SMO and whether this would indeed result in an increased content of desmethyl sterols. Tobacco is not a typical oil seed plant, and the results obtained in tobacco might not necessarily be valid in plants, which are not closely related. Furthermore, some of the claimed plants (e. g. rape, sunflower) have been extensively used and bred as oil seeds, i. e. with the intention to obtain plants with a high oil content, so that their metabolic pathways might not be easily further changed towards one specific group of sterols. Hence, the description does not fulfil the criteria of Article 5 PCT.